ION EXCHANGE PROPERTIES OF THE CANINE CAROTID ARTERY

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ABSTRACT Properties of the ion exchange mechanisms in the arterial wall were investigated by comparing water and electrolyte contents, and by measuring the steady-state entry of ^{22}Na, ^{42}K, and ^{36}Cl under similar in vitro conditions. Overnight incubation of freshly dissected slices at 2°C resulted in an accumulation of sodium, chloride, and water and a loss of potassium. Subsequent incubation at 37°C in a physiological solution resulted in a reversal of these processes. Loss of water, sodium, and chloride at 37°C could also take place into a potassium-free solution. Under all conditions studied the quantity of fast exchanging electrolyte (half time less than 3 min) exceeded that contained in the inulin and sucrose spaces. The excess could not be attributed to connective tissue adsorption. A kinetic model was applied to the flux data which incorporated two simultaneous processes: bulk diffusion and a reversible reaction. The assumption that the cell membrane behaved as a discrete barrier for the exchange of all cell electrolyte was relaxed in this approach. A theory based upon the physicochemical properties of proteins, ions, and water in biological systems provided a physical basis for the kinetic model, and for interpreting the ion exchange properties of the vascular wall.

INTRODUCTION

Ion exchange processes in the arterial wall are considered to play an important role in the control of its mechanical properties. Relatively few studies, however, have been conducted in the dynamic behavior of these processes in blood vessels. The complex structure of the vessel wall has made difficult the partitioning of electrolyte between cellular and extracellular components. To overcome this problem, studies involving the exchange of radioactive isotopes have been undertaken by several investigators (Barr, 1961; Briggs and Melvin, 1961: Garrahan et al., 1965; Hagemeijer et al., 1965; Jones et al., 1965; Llaurado et al., 1967; Keatinge, 1968; Rorive et al., 1967; Türker et al., 1967; Villamil et al., 1967; Villamil, Rettori, Barajas, and Kleeman, 1968; Villamil, Rettori, Yeyati, and Kleeman, 1968; Yeyati and Taquini, 1967). One approach taken was to measure the effect of vasoactive agents upon the isotope exchange (Barr, 1961; Briggs and Melvin, 1961; Rorive et al., 1967; Türker et al., 1967). Such agents were regarded as affecting only smooth muscle, and changes in flux were taken to indicate alteration in membrane per-

meability. It was usually assumed that the exchange was that of a two-compartment system separated by a rate-limiting membrane. Several investigations indicated that the ion exchange properties of the vascular smooth muscle may follow more complex kinetics with the existence of more than one compartment (Garrahan et al., 1965; Hagemeijer et al., 1965; Jones et al., 1965; Llaurado et al., 1967; Villamil et al., 1967; Villamil, Rettori, Barajas, and Kleeman, 1968; Villamil, Rettori, Yeyati, and Kleeman, 1968). The possibility arises that for ions such as sodium there may be relatively rapid (half time < 5 min) and slowly exchanging cell fractions. It has been noted that the quantity of sodium in the fastest exchanging component may exceed that in the inulin space (Garrahan et al., 1965; Villamil et al., 1967). Immobilization of ions by vascular connective tissue was regarded as a factor contributing to this observation (Garrahan et al., 1965). On the other hand, recent studies of chloride exchange indicated that immobilization to connective tissue was insufficient to account for the quantity of fast exchanging chloride in dog carotid artery (Villamil, Rettori, Yeyati, and Kleeman, 1968). Many uncertainties still exist, however, concerning the electrolyte exchange kinetics and distribution within the arterial wall.

The objective of the present study was to characterize basic properties of electrolyte exchange mechanisms in the arterial wall. Two approaches were taken: (1) comparisons were made between slices equilibrated under conditions designed to induce large shifts of water and electrolyte; and (2) under these conditions the steady-state entry of radioactive isotopes was measured. Some of the principal findings were interpreted in terms of a unified model for ion exchange processes.

METHODS

Mongrel dogs weighing 20–25 kg were killed by a captive bolt device. Both common carotid arteries were removed and placed in cold physiological salt solution with potassium and glucose omitted, and gassed with 3% CO_2 and 97% N_2. This procedure inhibited autolysis and led to the elimination of ion gradients. The artery was rinsed, trimmed of loose adventitia, cut along its length, and finally sliced into rectangular pieces with a stainless-steel razor blade. Each carotid artery yielded 12–20 slices approximately 0.7 mm thick and weighing 25–50 mg. Analysis of freshly sampled distal and proximal ends of the right and left common carotid artery (13 dogs) indicated no major site differences. Samples for fresh tissue analysis were removed from the arteries before exposure to the salt solution. The slices were incubated overnight at an average temperature of 2°C in the potassium- and glucose-free solution. The experimental incubations were carried out at 37°C or 2°C in a metabolic shaker rotated at 120–140 revolutions per minute. Incubation periods of 3 hr were used to insure a steady state for the electrolyte contents (Barr et al., 1962). After incubation, each slice was removed from the flask, blotted, and placed in tared vials for analysis. The blotting procedure consisted of placing the slice, adventitia side down, upon two pieces of Whatman No. 40 filter paper and of lightly stroking the intima and cut sides with the edge of another sheet of filter paper. This procedure took about 10–15 sec and was begun several seconds before the end of the desired incubation period. This transfer technique was standard for all experiments and was estimated to introduce a 3–5 sec error.

Solutions were prepared from distilled deionized water and analytical reagent grade salts. The incubation medium was similar to Krebs' solution, and following Bohr's suggestion will be termed physiological salt solution and abbreviated PSS (Bohr, 1964). The ion concentrations were: Na^+, 145 mM; K^+, 4.5 mM; Mg^{2+}, 1.2 mM; Ca^{2+}, 2.5 mM; Cl^-, 126.3 mM; HCO_3^-, 22.5 mM; SO_4^{2-}, 3.45 mM; $H_2PO_4^-$ + HPO_4^{2-}, 1.2 mM; and a glucose concentration of 5.5 mM. Solutions were gassed with 5% CO_2 and 95% O_2 at 37°C, and 3% CO_2 and 97% N_2 at 2°C. Metabolically inhibited conditions were achieved at 37°C by adding 95% N_2 in place of O_2; iodoacetate, 1.0 mM; 2,4-dinitrophenol, 0.2 mM; cyanide, 4.5 mM; and by the omission of glucose.

The water and electrolyte contents of the tissues were measured in the same manner as that previously reported (Jones et al., 1964). The water content was determined after oven drying for 24 hr at 95°C. The electrolytes were extracted into 3 ml of 0.1 N HNO_3 and 225 ppm Li (internal standard). Sodium and potassium were analyzed on a flame photometer, and chloride on a chloride titrator. The total electrolyte per slice was corrected for the quantity contained in blank vials (about 5% for sodium and 2% for potassium and essentially zero for chloride). Tissue water was expressed per kilogram wet weight (kg wet wt) and per kilogram dry solid (kg d.s.). The electrolyte was expressed per kilogram dry solid. The dry-solid representation was considered to yield the more stable basis for comparison of shifts of water and electrolyte.

For the inulin space and sucrose space measurement, the slices were incubated in medium containing 0.75% inulin or 0.5% sucrose for a period of 3 hr. This time has been noted to be sufficient to establish a steady state in the vessel wall (Norman et al., 1959; Villamil, Rettori, Barajas, and Kleeman, 1968). Saline extracts of the slices were subsequently analyzed for inulin and sucrose by a resorcinol method (Higashi and Peters, 1950). The inulin space was expressed per kilogram wet weight and per kilogram dry solid. The total water content was measured in duplicate slices. Since sufficient slices were not available in the experiments in which sucrose distribution was measured, the wet weight representation only was used.

A modification of the routine procedure was employed in the study of isotope uptake. Slices were incubated under the experimental conditions for 3 hr to insure steady-state conditions after cold storage. The slices were then transferred into identical medium containing one of the following isotopes: ^{22}Na, 0.1 μc/ml; ^{42}K, 0.5–1.0 μc/ml; or ^{36}Cl, 0.1 μc/ml. The incubations continued for periods from 30 sec until greater than 99% exchange was obtained, or in one series 48 hr (PSS + ^{22}Na, 2°C).

The ^{22}Na and ^{36}Cl were added directly to the prepared physiological salt solutions. In the case of ^{42}K, because significant amounts of carrier were present (70–170 mc/g K), appropriate amounts of KCl were omitted to maintain the final K concentration at 4.5 mM. The volume of medium in the incubation flask was kept large in relation to the tissue volume to maintain a constant external specific activity.

Tissues incubated in ^{22}Na were counted along with standards prepared from the bath solution after the dried specimens were extracted into 3 ml of the flame photometry solution. Because ^{42}K has a relatively short half-life (12.45 hr), it was necessary to extract the weighed tissues into distilled water for counting about 1 hr after the end of the incubation period. The tissue and added water were subsequently dried to determine the dry weight. Activity in the samples was corrected for the radioactive decay which took place between the time of their counting and that of the standards. A Baird-Atomic gamma counting system was employed. In the case of ^{36}Cl, dried tissue and standards were extracted into 3 ml of the flame photometry solution. 2 ml was placed in 18 ml of Bray's solution for counting in a liquid scintillation spectrometer (Packard Tri-Carb) (Bray, 1960).

Tissue chloride content was determined on 0.5 ml of the remaining acid extract. Total

tissue sodium and potassium were determined by flame photometry. The electrolyte contents were also determined on the standards and routinely were found to be within 2% of the calculated values. From the count ratio of the tissue and standard activities, the quantity of electrolyte exchanging with the isotope was determined for each slice. The fraction exchanged was calculated by dividing this value by the total amount of measured electrolyte, and has been designated: $^{22}Na/Na_T$ for sodium; $^{42}K/K_T$ for potassium; and $^{36}Cl/Cl_T$ for chloride.

RESULTS

In order to characterize the ion exchange properties of the arterial wall, tissue water and electrolyte contents were evaluated under conditions chosen to result in maximal shifts of these constituents. The values are summarized in Table I. Segments were sampled immediately after death (fresh) and after incubation for 20–24 hr at 2°C in potassium- and glucose-free medium with subsequent incubation at: 2°C in normal solution (PSS, 2°C); 37°C in potassium-containing medium (PSS, 37°C); 37°C in potassium-free medium (PSS−K, 37°C); and 37°C under inhibited conditions (Inhib., 37°C). Large shifts of water and electrolyte into fresh segments were induced by incubation. These were largest under cold storage, which resulted in swelling of the arterial wall coupled with accumulation of sodium and chloride and loss of potassium ($P < 0.001$). Over 97% of the initial potassium content could be extracted into PSS−K at 2°C. This process was reversible, as indicated by the alteration in water and electrolyte at 37°C in PSS. The shifts were characterized by a loss of water, sodium, and chloride ($P < 0.001$), and by an accumulation of potassium ($P < 0.001$). Comparison of the inulin space (dry weight basis) in PSS, 2°C and PSS, 37°C indicated that the loss of water and electrolyte was from the water not permeable to inulin ($P < 0.001$). The ion shifts which take place under such conditions can be described in terms of a net movement of sodium chloride and water out of the arterial wall coupled with a sodium-potassium exchange. Similar shifts have been noted in other smooth muscles (Buck and Goodford, 1966; Kao and Siegman, 1963; Armstrong, 1964) and tissues in general (Robinson, 1960).

The composition of the fresh tissue was approached in PSS, 37°C, but was not achieved. The incubated slices contained more water, sodium, and chloride than the fresh samples ($P < 0.001$). The accumulation of potassium to within 90% of the fresh value indicated that the function of the smooth muscle was not markedly impaired. Increased water and electrolyte could have been the result of an expanded extracellular space. The inulin spaces reported for freshly dissected carotid arteries were less than in PSS, 37°C (Jones et al., 1964; Villamil, Rettori, Barajas, and Kleeman, 1968). The in vivo evaluation of the extracellular space would be helpful in clarifying this point.

The role of potassium in the recovery process was evaluated by transferring slices directly from PSS−K, 2°C to PSS−K, 37°C. The water, inulin space, sodium, and chloride contents in PSS−K, 2°C were equivalent to those in PSS, 2°C given in Table I. The change in temperature resulted in a loss of water and equivalent

TABLE I

EFFECT OF INCUBATION ON WATER AND ELECTROLYTE CONTENTS

Treatment	No. of dogs	Water	Inulin space	Water	Inulin space	"Cell" water	Total electrolyte			"Cell" electrolyte		
							Na	K	Cl	Na	K	Cl
		g/kg wet wt		*kg/kg d.s.*			*meq/kg d.s.*			*meq/kg d.s.*		
Fresh	19	719	—	2.57	—	—	310	135	247	—	—	—
		±2		±0.03			±7	±3	±4			
PSS, 2°C	17	800	342	4.00	1.72	2.31	617	23	473	367	15	256
		±2	±12	±0.06	±0.06	±0.06	±9	±1	±7	±8	±1	±9
PSS, 37°C	28	759	420*	3.17	1.74*	1.39	408	123	349	156	116	129
		±3	±9	±0.05	±0.05	±0.04	±9	±4	±7	±7	±4	±6
PSS-K, 37°C	18	739	342‡	2.85	1.35‡	1.58	457	2	309	261	2	145
		±4	±13	±0.08	±0.07	±0.06	±10	±1	±8	±9	±1	±7
Inhib., 37°C	10	777	—	3.48	—	—	532	18	417	—	—	—
		±3		±0.05			±7	±1	±7			

± Standard error of mean.
* 24 dogs.
‡ 15 dogs.

amounts of sodium and chloride ($P < 0.001$). About one-third of this loss could be attributed to a decrease in the inulin space ($P < 0.001$); however, a significant decrease of the water not permeable to inulin and related sodium and chloride also occurred ($P < 0.001$). For convenience this material has been designated "cell" water or "cell" electrolyte in Table I although some noncellular material may be included (see below). The net movement of sodium chloride and water from vascular smooth muscle could therefore take place independently of a coupled sodium-potassium exchange. This finding agrees with the observation of Friedman et al. (1968) that the tail artery of the rat could lose sodium into a "Na-free, Tris" solution without a coupled uptake of potassium.

The dependence of the water and electrolyte exchange processes at 37°C on a functional energy metabolism was studied by exposing slices to inhibitors of glycolysis and oxidative metabolism (Inhib., 37°C). Under these conditions the recovery from cold storage was essentially blocked. There was some loss of water and electrolyte ($P < 0.001$), however, which may have been the result of nonspecific temperature effects upon the vascular wall.

Although the changes in calculated "cell" electrolyte, presented in Table I, were taken as being indicative of smooth muscle exchange, there may be some question as to the absolute quantity associated with the cellular component. It is common practice to partition the electrolyte simply between extracellular components (estimated here by the inulin space) and cellular components (estimated by the total minus that relegated to the inulin space). This may be an oversimplification for the vascular wall, which is relatively rich in connective tissue (Harkness et al., 1957; Fischer and Llaurado, 1966). The problem of ion sequestration by connective tissue was approached by analyzing the water and electrolyte contents of adventitia trimmed from the carotid slices; the results are presented in Table II. The difference between the electrolyte measured by tissue analysis and that calculated from the total water content times the concentration of electrolyte in the incubation medium was taken as the sequestered electrolyte. For the three ions measured, the differences were relatively small. On the basis of a nonpaired Student t test, the differences between measured and calculated values were not significant. On the basis of a paired test, the sodium difference was significant ($P < 0.001$) and that for potassium suggestive ($P = 0.01$). It would appear, however, that ion sequestering to the connective tissue making up the adventitia (mostly collagen) was a minor factor in the accumulation of ions by the arterial wall.

In order to determine the range of values which might be relegated to the extracellular water in the arterial wall, the distribution of sucrose and inulin was compared on randomly mixed slices from three dogs and is summarized in Table III. The sucrose space for each of the three treatments was greater than the corresponding inulin space ($P < 0.001$). Therefore the quantity of water and electrolytes calculated to be in the cellular component depended critically upon the size of the molecule used to estimate the extracellular space.

TABLE II

COMPOSITION OF ADVENTITIA

Treatment	No. of dogs	Total water		Measured electrolyte			Calculated electrolyte		
				Na	K	Cl	Na	K	Cl
		g/kg wet wt	*kg/kg d.s.*	*meq/kg d.s.*					
PSS, 37°C	12	859 ±12	6.66 ±0.71	949 ±99	—	—	966	—	—
PSS, 37°C	7	821 ±11	4.75 ±0.44	—	23.8 ±2.4	—	—	21.3	—
PSS, 37°C	22	861 ±10	6.78 ±0.46	—	—	860 ±56	—	—	856

± Standard error of mean.

TABLE III

COMPARISON OF INULIN AND SUCROSE DISTRIBUTION

Treatment	Inulin space	Sucrose space	Ratio sucrose/inulin
	g/kg wet wt		
PSS, 2°C	329 (11)* ±10	492 (11) ±19	1.50
PSS, 37°C	424 (12) ±6	563 (12) ±15	1.33
PSS − K, 37°C	377 (10) ±10	521 (12) ±17	1.38

± Standard error of mean.

* Number of slices.

Dynamic aspects of the ion exchange properties of the arterial wall were studied by measuring the steady-state entry of radioactive isotopes. This approach was expected to yield information concerning the rate of turnover as well as the relative magnitude of the exchanging components. One approach taken was to treat the isotope exchange as a series of exponentials with the following relation:

$$\left(1 - \frac{b}{b_T}\right) = f_1 e^{-k_1 t} + f_2 e^{-k_2 t} + \cdots$$

where $(1 - b/b_T)$ = 1 minus the fraction exchanged during the influx experiment; f = fraction of total; k = rate constant; t = time.

This approach assumed uniform mixing within each compartment, with a sharply localized resistance to flow between compartments.

To facilitate analysis, the data were plotted as log (1−fraction exchanged) versus time. The time constants and intercepts were derived from such plots in a

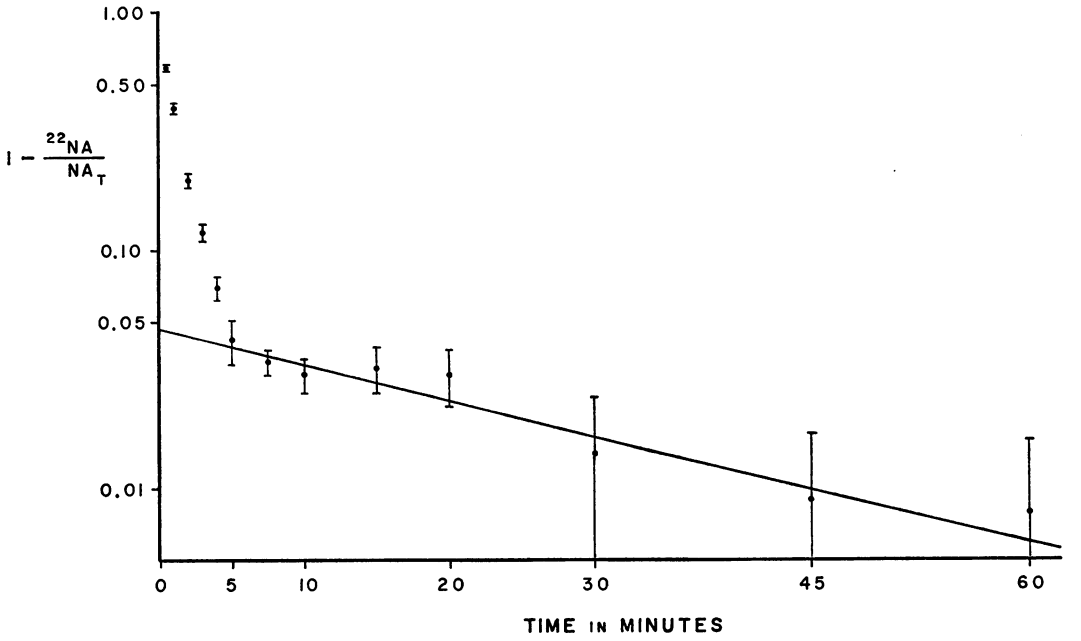

FIGURE 1 Steady-state exchange of tissue sodium (Na_T) with radioactive ^{22}Na physiological salt solution at 37°C. Abscissa, time in minutes of incubation in radioactive solution. Logarithmic ordinate scale, average values (7 dogs) for 1 minus fraction of tissue sodium exchanged. Vertical bars represent ±1 standard error of mean. Straight line represents a single exponential function fitted to the slowly exchanging component.

manner described by Solomon (1960). This consisted of fitting a straight line to the end of the curve and extrapolating it to zero time to determine the intercept. This line was subtracted from the experimental data and the procedure was repeated. Two exponentials were fitted to most of the flux curves. The fast portion (half-time less than 3 min) is usually considered to characterize the extracellular exchange, while the slow fraction is associated with cellular components.

Average values for ^{22}Na flux in PSS, 37°C appear in Fig. 1. More than 95% of the tissue sodium was characterized by a rapid exchange. In the case of ^{36}Cl under similar conditions, over 90% exchanged rapidly; the values are shown in Fig. 2. The exchange of ^{42}K under physiological conditions differed greatly from that of sodium and chloride and is shown in Fig. 3. Only a small portion of the total potassium exchanged rapidly, while the predominant component exchanged slowly. This component exhibited some deviation from a single exponential (straight line, Fig. 3). The small departures in absolute terms could be accommodated by treating the potassium exchange constant as a distributed parameter. The application of this approach to ^{42}K exchange has been described by Creese et al. (1956). The mean can be derived from the time required to reach $1/e$ of the initial amount. This value

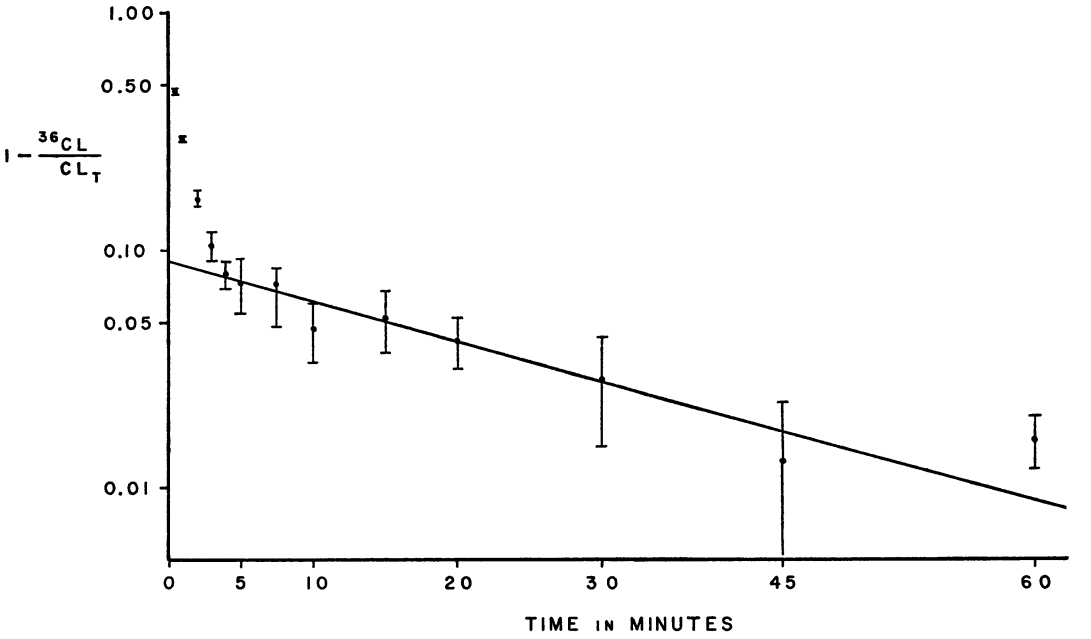

FIGURE 2 Steady-state exchange of tissue chloride (Cl_T) with radioactive ^{36}Cl physiological salt solution at 37°C. Plotted as in Fig. 1 with average values (6 dogs ±,1 standard error of mean.

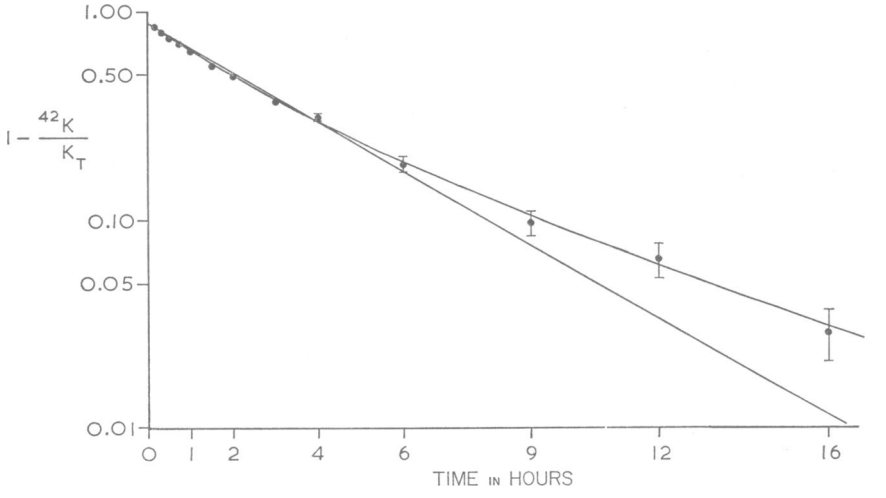

FIGURE 3 Steady-state exchange of tissue potassium (K_T) with radioactive ^{42}K physiological salt solution at 37°C. Plotted as in Fig. 1 (time in hours) with average values (9 dogs) ±1 standard error of mean. Total potassium contents were unaffected by the time of incubation in the isotope. The curve was calculated on the basis of a log normal distribution (standard deviation = 0.5 log unit) for the exchange constant applied to the single exponential function.

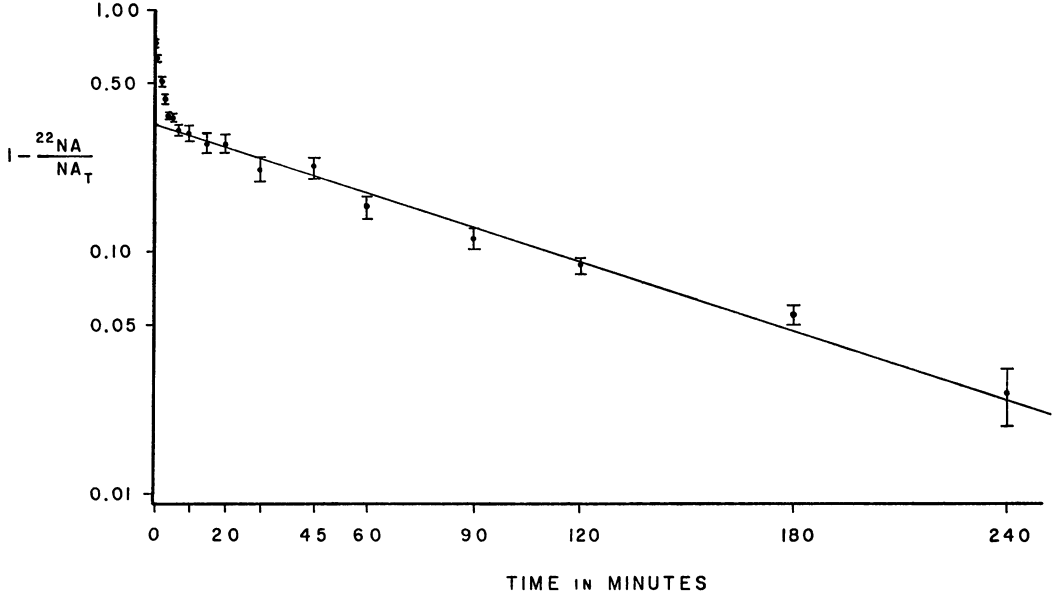

FIGURE 4 Steady-state exchange of tissue sodium with radioactive ^{22}Na potassium-free salt solution at 37°C. Plotted as in Fig. 1 with average values (6 dogs) ±1 standard error of mean.

agrees with the time constant for the single exponential as indicated by the intersection in Fig. 3. The standard deviation for the log normal distribution was obtained by comparing experimental results with computed curves (Creese et al., 1956) adjusted for the 12% fast fraction. A value of 0.5 yielded a reasonable fit and fell between that applied to mammalian skeletal muscle (Creese et al., 1956) and to cardiac muscle (Persoff, 1960).

It was noted above that PSS−K at 37°C resulted in an increased "cell" sodium and decreased "cell" potassium content in comparison with PSS, 37°C. The entry of ^{22}Na was measured under potassium-free conditions and is represented in Fig. 4. The portion of tissue sodium characterized by a slow exchange process was markedly increased and accounted for about one-third of the total sodium.

The ^{22}Na entry was also studied under two conditions of metabolic inhibition. Average values for the flux in PSS, 2°C and Inhib., 37°C are shown in Fig. 5. At 2°C, 30% of the sodium exchanged slowly, whereas no such component was apparent under inhibited conditions at 37°C.

The derived intercepts and rate constants for all the isotope exchange experiments

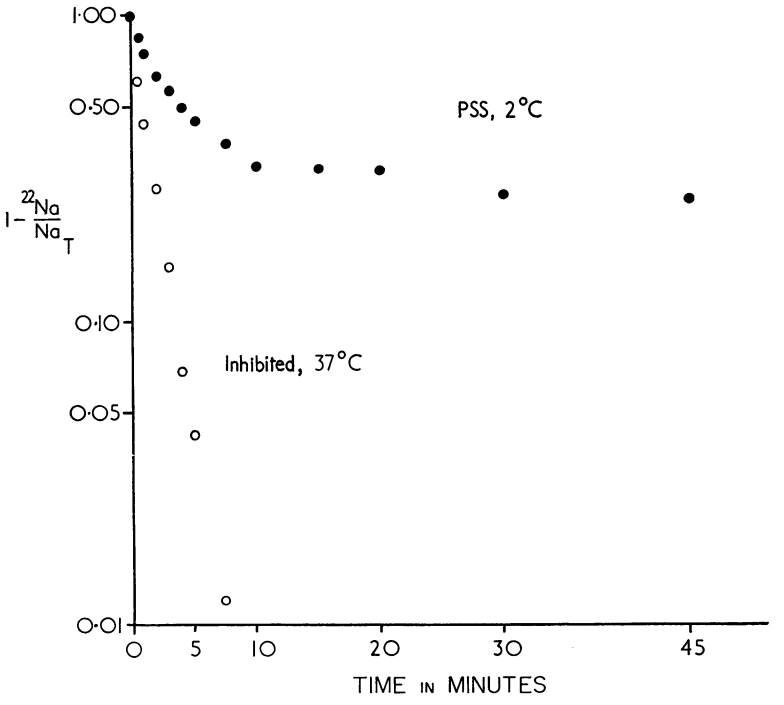

FIGURE 5 Steady-state exchange of tissue sodium with radioactive ^{22}Na physiological salt solution at 2°C (solid circles) and with ^{22}Na under metabolically inhibited conditions at 37°C (open circles). Plotted as in Fig. 1 with average values at 2°C (3 dogs) and 37°C (2 dogs).

TABLE IV

VALUES FOR EXPONENTIAL EQUATION AND ELECTROLYTE DISTRIBUTION

Treatment	No. of dogs	Intercepts		Rate constants		Electrolyte distribution			
		f_1	f_2	k_1	k_2	f_1	f_2	Inulin space	Sucrose space
				min^{-1}		*meq/kg d.s.*			
^{22}Na									
PSS, 2°C	3	0.70	0.23*	0.26	0.1×10^{-2}	432	185‡	249	374
PSS, 37°C	7	0.95	0.05	0.92	3.5×10^{-2}	388	20	252	335
PSS − K, 37°C	6	0.67	0.33	0.69	1.1×10^{-2}	307	150	196	270
Inhib., 37°C	2	1.00	—	0.69	—	532	—	—	—
^{42}K									
PSS, 2°C	2	0.60	0.40	0.63	0.9×10^{-2}	14	9	8	12
PSS, 37°C	9	0.12	0.88	1.39	0.45×10^{-2}	15	108	8	10
Inhib., 37°C	2	1.00	—	1.00	—	18	—	—	—
^{36}Cl									
PSS, 2°C	3	0.80	0.20	0.69	2.3×10^{-2}	378	95	217	326
PSS, 37°C	6	0.91	0.09	1.16	3.8×10^{-2}	318	31	220	292

* 0.07 exchanged with $k = 4 \times 10^{-6}$ min^{-1}.

‡ Includes both slow fractions.

are summarized in Table IV. Values are also given for the quantity of electrolyte associated with fast and slowly exchanging fractions. These values were calculated by multiplying the fraction associated with each component by the total electrolyte (Table I). The total electrolyte contents of the slices used in the flux studies were essentially the same as these values. The electrolyte contained in the inulin space was calculated by multiplying the inulin space (Table I) by the extracellular concentration (see Methods). The sucrose space electrolyte was calculated by taking the ratio of sucrose distribution to inulin distribution (Table IV) times the electrolyte calculated to be in the inulin space.

Under every experimental condition investigated, the quantity of fast exchanging electrolyte was greater than that associated with the sucrose space, which in turn exceeded that in the inulin space. If a correction were applied to the exchange data for exponential interaction, the discrepancy between fast exchanging electrolyte and that in the saccharide space would be increased (Huxley, 1960). The appearance of excess fast exchanging electrolyte in the presence of slowly exchanging components underscored the complex nature of the exchange processes. A kinetic model based on the cell membrane behaving as a discrete barrier to electrolyte exchange with homogeneous phases on both sides appears to be too simple.

An alternative kinetic model was applied to the flux data. The model incorporated two simultaneous processes: bulk diffusion of the isotope into a plane sheet; and adsorption-desorption which for simplicity was assumed to be first order. This problem

has been treated by Crank (1956) and its solution has been applied to permeability properties of some tissues other than smooth muscle (Ling et al., 1967; Ling, 1966). Bulk diffusion is governed by the equation

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - \frac{\partial S}{\partial t} \tag{1}$$

with the simultaneous reaction of the type

$$\frac{\partial S}{\partial t} = \lambda C - \mu S \tag{2}$$

where D = diffusion coefficient; λ = rate constant for forward reaction; μ = rate constant for backward reaction; C = concentration of solute free to diffuse; S = concentration of immobilized solute; x = space coordinate perpendicular to the sheet, a is the half thickness, and $-a \leq x \leq a$.

The buildup of material in the extracellular space and cell water containing rapidly exchanging electrolyte (defined as the interstitial water) would take place by diffusion. The assumption that a discrete resistance which limits the exchange of all cell electrolyte exists at the cell surface or membrane was relaxed in the application of this model. Simultaneously material would accumulate into a nondiffusing phase via a first-order reaction (λC), but would also break down via a backward reaction (μS). At equilibrium both processes would be equal (when $\partial S/\partial t = 0$, $\lambda/\mu = S/C = R$, the distribution constant).

If the membrane were treated as a discrete resistance, it would be necessary to incorporate a proportionality constant (about 0.6) into the diffusion term to represent the fraction of the tissue available for a bulk diffusion process. In addition, a second adsorbed fraction characterized by relatively fast exchange kinetics could be inserted into equation (1). It is anticipated that with the additional parameters the agreement between theory and experiment would be as good as that of the preceding paragraph. The model in which the fast exchanging electrolyte is free to diffuse throughout the bulk of the tissue was preferred on grounds of economy, because fewer parameters were optimized in its application.

The Crank-Nicolson method, applied with finite difference approximation of the differential equations, was used in the numerical analysis (Appendix, Crank, 1956). Equations (1) and (2) were integrated over distance and time. The thickness ($2a$) of the slices was measured under a dissection microscope with a calibrated ocular and averaged 0.7 mm (standard deviation = 0.2 mm). The values for μ were estimated from the rate constants for the slowly exchanging fraction (Table IV) and the values for R were calculated from the ratio of the intercepts, $R = f_2/f_1$ (Table IV). The values for the free diffusion coefficient for each ion were used as a starting point, but the best fits were achieved by trial and error using somewhat lower values.

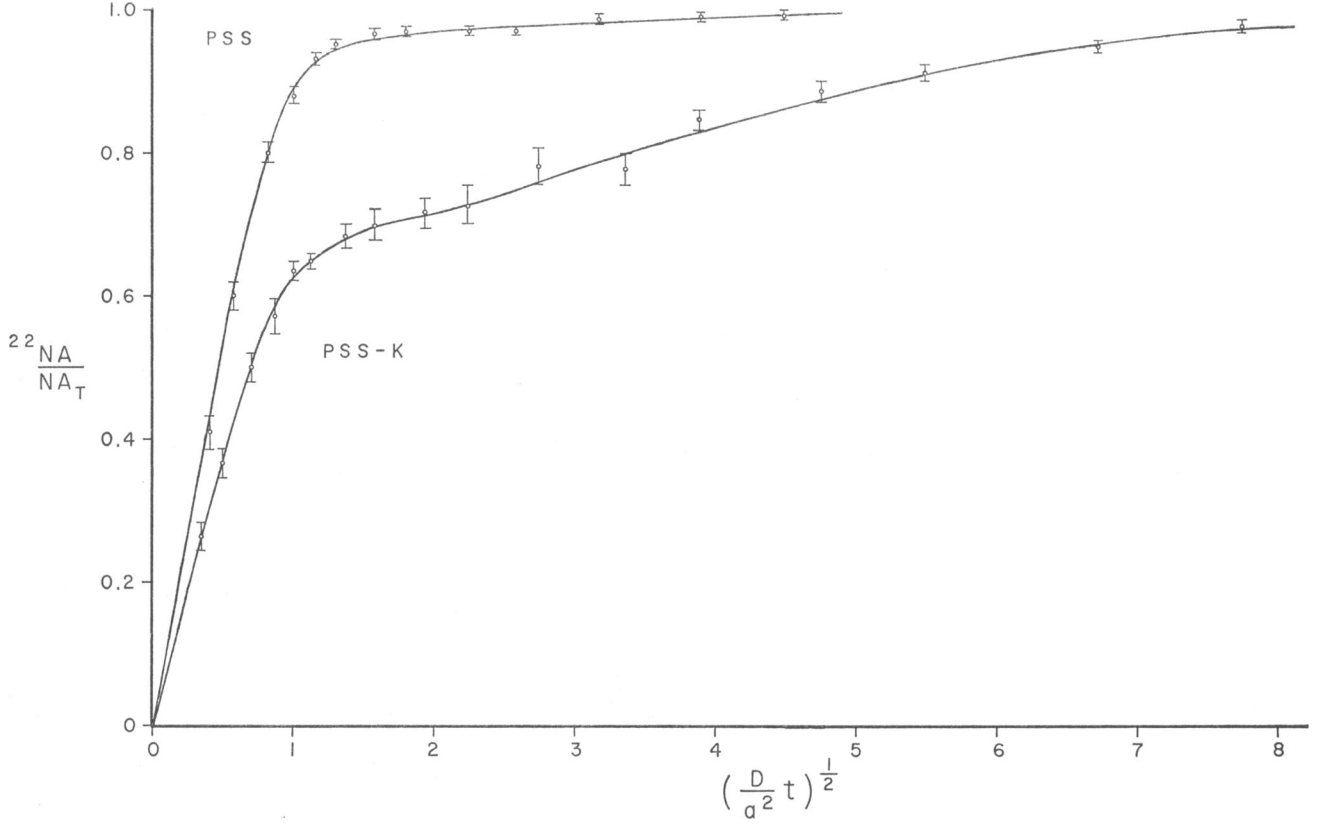

FIGURE 6 Steady-state exchange of tissue sodium with ^{22}Na physiological salt solution (PSS) and potassium-free salt solution (PSS − K) at 37°C. Abscissa, nondimensional time parameter. Ordinate, fraction exchanged, replotted from Fig. 1 and Fig. 4. Theoretical curves computed from equations (1) and (2) using parameters given in Table V.

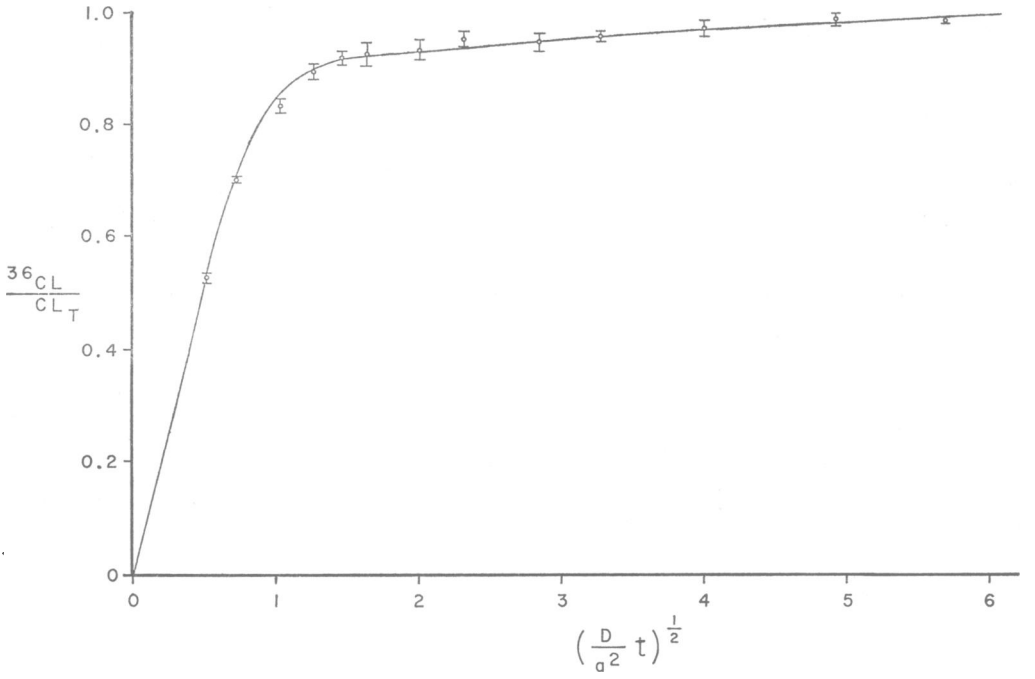

FIGURE 7 Steady-state exchange of tissue chloride with ^{36}Cl physiological salt solution at 37°C. Plotted as in Fig. 6, experimental values replotted from Fig. 2. Theoretical curve computed from equations (1) and (2).

The calculated curves and experimental values for ^{22}Na entry in PSS and PSS−K at 37°C are plotted in Fig. 6. The fraction exchanged is plotted on the ordinate and values for $(D^t/a^2)^{1/2}$ are plotted along the abscissa. This plot was in accordance with the practice of Crank in using nondimensional parameters for the graphs (Crank, 1956). Both sets of data were in good agreement with the calculated curves. The values for ^{36}Cl exchange are shown in Fig. 7, and good agreement was found between the model and experimental values. The predominant component for the sodium and chloride exchange was that described by bulk diffusion kinetics.

In the case of ^{42}K in PSS, 37°C, there was a shoulder corresponding to the time at which diffusion limited exchange reached equilibrium; it is shown in Fig. 8. Under inhibited conditions only the diffusion limited component for ^{42}K entry was apparent (the same observation applied to ^{22}Na under inhibited conditions at 37°C). The uptake data for potassium under inhibited conditions were normalized on the basis of the total potassium content measured under supported conditions. The effect of metabolic inhibition was therefore to remove the tissue component which followed reversible reaction kinetics.

The constants employed in the calculation of the theoretical curves are summarized in Table V. The values for the diffusion coefficients were of the same order

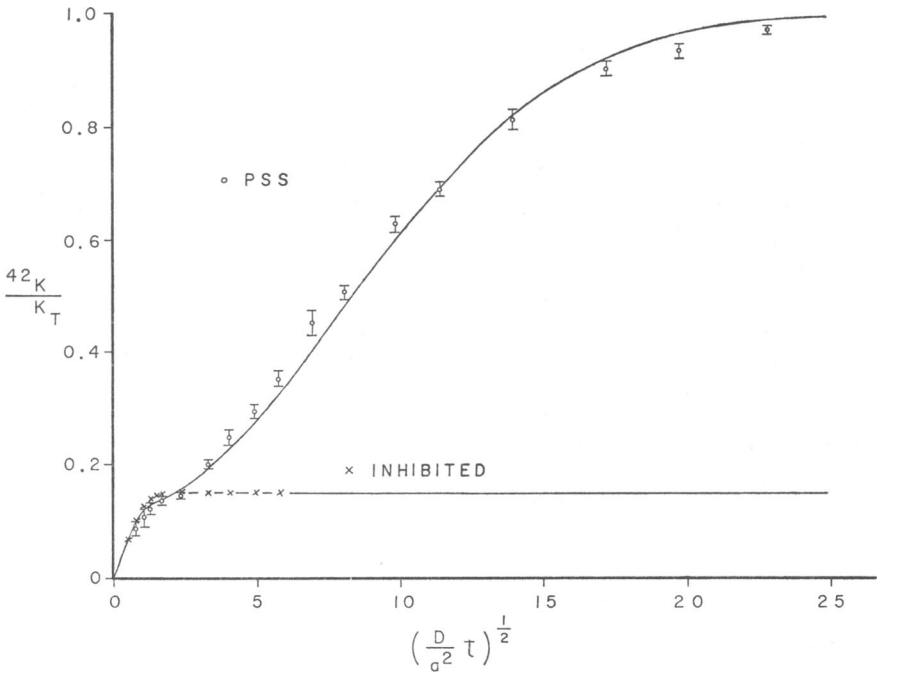

FIGURE 8 Steady-state exchange of tissue potassium with ^{42}K physiological salt solution (open circles, PSS) and with ^{42}K under metabolically inhibited conditions (crosses, Inhibited) at 37°C. Plotted as in Fig. 6, experimental values replotted from Fig. 3 (PSS) and average values (2 dogs) for inhibited conditions. Theoretical curve computed from equations (1) and (2). Straight line represents fraction following bulk diffusion kinetics.

TABLE V
CONSTANTS FOR BULK DIFFUSION—REVERSIBLE CHEMICAL REACTION EQUATION

Treatment	R	D	D/a^{2*}	μ	$D/a^2\mu$
		$cm^2\ sec^{-1}$	sec^{-1}	sec^{-1}	
^{22}Na					
PSS, 37°C	0.05	0.7×10^{-5}	5.6×10^{-3}	5.6×10^{-4}	10
PSS − K, 37°C	0.50	0.5×10^{-5}	4.2×10^{-3}	1.8×10^{-4}	23
^{42}K					
PSS, 37°C	7.0	1.1×10^{-5}	9.0×10^{-3}	7.5×10^{-5}	120
^{36}Cl					
PSS, 37°C	0.1	1.1×10^{-5}	9.0×10^{-3}	6.4×10^{-4}	14

* a = 0.035 cm.

of magnitude as those for free diffusion. Since the radius of vascular smooth muscle cells (about 2 μ) is small (Rhodin, 1962) in comparison with the half thickness of the slice (a = 350 μ), the time constant for any interstitial diffusion through the cell water could be as much as five orders of magnitude less than that applied to the slice.

DISCUSSION

In these studies it was noted that there is a larger quantity of fast exchanging electrolyte in the vascular wall than can be accommodated in the inulin or sucrose space. The turnover of relatively large amounts of sodium and chloride appeared to be limited by bulk diffusion. Slowly exchanging fractions which followed a first-order reversible reaction were also present. Under physiological conditions potassium was the predominant constituent, whereas under potassium-free conditions at 37°C a similar amount of sodium exchanged slowly.

The association of the slowly exchanging electrolyte with the cellular components can be established by its disappearance under conditions of metabolic inhibition at 37°C. The location of nearly all this material in smooth muscle can be inferred from the small contribution of the endothelial cells and fibroblasts to the total cell mass (Rees, 1968; see below).

In order to establish the probable intracellular location of much of the fast exchanging electrolyte held in excess of that in the saccharide spaces, it will be necessary to review mechanisms proposed for the extracellular location of such electrolyte. One premise of these mechanisms is that the cell membrane behaves as a discrete barrier for the exchange of all or almost all the intracellular ions. Recent investigations into the sodium-potassium exchange properties of the rat tail artery and aorta led Friedman and coworkers to conclude that about 10 meq sodium per 100 g dry solid (100 meq/kg d.s.) was loosely bound or trapped in a "paracellular matrix" (Friedman and Friedman, 1967; Friedman, Gustafson, Hamilton, and Friedman, 1968; Friedman, Gustafson, and Friedman, 1968). This conclusion was based on evidence that the quantity of sodium which was readily extracted into low-sodium solutions exceeded that contained in the inulin space. Inulin was considered by these investigators to distribute into the "free interstitial fluid." Similar results were found at low temperatures, indicating that metabolic processes were not involved.

A comparison of inulin distribution and sodium exchange properties of freshly dissected dog carotid indicated an excess of 100 meq/kg d.s. (Garrahan et al., 1965). Analysis of the distribution of sodium in dog carotid with disrupted cells (Headings et al., 1960) or under metabolically inhibited conditions (Villamil, Rettori, Barajas, and Kleeman, 1968) indicated that about 120 meq Na per kg d.s. were in excess of that dissolved in tissue water. Both these groups of workers suggested that these findings could be the result of binding to polyanions in the extracellular phase. The finding in the present study of 136 meq/kg d.s. of fast exchanging sodium in excess of that in the inulin space (PSS, 37°C) is in general agreement with the previous findings.

The concept of a gel-like extracellular phase which takes up sodium and holds water, but excludes relatively large molecules such as inulin, is a useful one. Although it appears to have some applications in the interpretation of electrolyte distribution and exchange properties of the arterial wall, the question is raised whether

such a matrix has the capacity to adsorb and/or dissolve the quantity of sodium required under the conditions tested.

One mechanism of accumulation in an extracellular matrix is adsorption onto polyanions making up collagen fibers prevalent in the adventitia or mucopolysaccharides prevalent in the media (Headings et al., 1960). On the basis of adventitia analysis in the present study, sequestering of sodium to this component can be neglected as a factor. The mucopolysaccharide contribution to sodium immobilization can be estimated from data available in the literature. The acid mucopolysaccharide chondroitin sulfate has been implicated in electrolyte immobilization processes (Farber and Schubert, 1957; Farber, 1960). Acid-hydrolyzable sulfate has been regarded as being a measure of this material in the arterial wall (Kirk, 1959). Each repeating unit or period of chondroitin sulfate contains one carboxyl and one sulfate group which may be associated with a counter ion such as sodium or calcium. Although two sites are available, on the average 0.85 mole of sodium was immobilized per period or sulfate (Farber and Schubert, 1957). Analysis of human aorta yielded values of 2.70 g/kg d.s. or 27.8 mmoles/kg d.s. for acid-hydrolyzable sulfate (Kirk and Dyrbe, 1956). The same analysis indicated the hexosamine content to be similar to that measured in dog carotid (Headings and Rondell, 1964). From these considerations the immobilized sodium in dog carotid would be:

$$\frac{24 \text{ mmoles Na}}{\text{kg d.s.}} \left(\frac{0.85 \text{ mmole Na}}{\text{mmole } SO_4} \times \frac{27.8 \text{ mmoles } SO_4}{\text{kg d.s.}} \right).$$

This value is considerably less than experimental values for the excess rapidly exchanging sodium, and the sodium postulated to be extracellularly bound under inhibited conditions.

Another approach to the problem of extracellular adsorption is noted in the following paper (Jones and Karreman, 1969), in which the quantity of fast exchanging potassium outside the sucrose space is determined as a function of the external concentration. Saturation behavior was found which is consistent with an adsorptive process with a maximum capacity of 11 meq/kg d.s. This again indicates an insufficiency of adsorptive sites.

A second possibility is that sodium may be dissolved in connective tissue water which excludes inulin. This water may be estimated by several means. First, measurements available for the connective tissue content of dog carotid indicate that collagen plus elastin make up 71 % of the dry solids (Fischer and Llaurado, 1966). An estimate of the associated water under present in vitro conditions can be taken from the water content (74 % wet weight or 2.8 kg H_2O per kg d.s.) of rabbit tendon incubated 24 hr and blotted similarly (Brading and Jones, 1969). Taking 71 % of the 2.8 kg H_2O per kg d.s. yields 2.0 kg H_2O per kg d.s. of connective tissue water in dog carotid. This represents an excess over the inulin space of 0.26 kg H_2O per kg d.s., accounting for an additional 40 meq Na per kg d.s.

A second means of estimating the connective tissue water is to accept the sucrose distribution as being throughout the extracellular water without significant penetration of the smooth muscle component. Because of evidence that sucrose can permeate at least one type of smooth muscle (Bozler and Lavine, 1958; Bozler, 1967), the authors are cautious about taking the sucrose distribution as entirely extracellular under the conditions employed. A comprehensive investigation of the extracellular space in freshly incubated dog carotid conducted by Villamil, Rettori, Barajas, and Kleeman (1968) demonstrated good agreement between the water associated with the anatomical space in electron micrographs and sucrose distribution. The latter exceeded the inulin space by 4–8% of the wet weight or 0.13–0.27 kg H_2O per kg d.s. (Table 1 in Villamil, Rettori, Barajas, and Kleeman, 1968). This can account for 20–40 meq/kg d.s. of dissolved sodium. Additional evidence that sucrose distribution was stable from 0.5 to at least 3 hr incubation in Krebs solution, and approached the total water content under metabolically inhibited conditions, gave further support to the value of the sucrose distribution as an extracellular marker (Villamil, Rettori, Barajas, and Kleeman, 1968). The mechanism proposed by Goodford and Leach (1966) for exclusion of large saccharides from water associated with mucopolysaccharides does not appear to be operative in the vascular wall, in that the distribution of inulin was unaltered by enzymatic digestion of this material (Villamil, Rettori, Barajas, and Kleeman, 1968).

The sucrose space could accommodate 83 meq Na per kg d.s. above that in the inulin space (Table IV: PSS, 37°C). Adding this to that associated with mucopolysaccharide still leaves about 30 meq/kg d.s. of fast exchanging sodium unaccounted for. Although the concept of a "paracellular matrix" present in the arterial wall is quite useful, it does not provide a complete basis for quantitative characterization of electrolyte distribution and exchange properties.

Another source of the heterogeneity in the distribution of fast exchanging sodium could lie in the variety of cell types in the vascular wall. Anatomical evidence, however, indicates that the carotid endothelial cells make up a very small portion of the cell mass and that the few fibroblasts are limited to the adventitia (Rees, 1968). Analysis of this latter component in the present study underscored its relatively acellular nature. Cell types other than smooth muscle would be expected to contribute only a few per cent to the cellular exchange, whereas the fast and slowly exchanging cellular components are of the same order of magnitude.

From the evidence available it appears that the smooth muscle component of the vascular wall is characterized by heterogeneous exchange of electrolyte. Although most of the attention has focused on sodium, both fast and slow exchange components are present for chloride. This confirms a recent finding that the cellular chloride exchange in dog carotid includes a fast exchanging component (Villamil, Rettori, Yeyati, and Kleeman, 1968). The quantity of excess fast exchanging potassium is relatively small. It will probably be necessary to make simultaneous measure-

ments of the extracellular space and ^{42}K exchange to characterize this component sufficiently.

Investigations carried out on other smooth muscles also indicate that under physiological conditions the cellular electrolyte does not exchange uniformly. It has been noted in frog stomach smooth muscle that the quantity of fast exchanging ^{42}K exceeded that contained in the inulin space (Bozler et al., 1958). The percentage of the tissue water available to the fast entry of ^{22}Na in toad stomach muscle was also found to exceed that available for the extracellular tracer, ^{131}I-labeled albumin (Burnstock et al., 1963). In the same study the kinetics of the ^{22}Na efflux also indicated a heterogeneous exchange of cell sodium.

Electrolyte exchange in the taenia coli of the guinea pig has been studied in some detail. In an early series of studies it was noted that at 35°C the quantity of ^{24}Na entering the strip within 5 min exceeded that contained in the extracellular space (Goodford and Hermansen, 1961; Freeman-Narrod and Goodford, 1962). Efflux studies also indicated that sodium was not simply distributed into a two-compartment system (Goodford, 1962). Investigation of ^{36}Cl exchange in the taenia coli also indicated that the cellular turnover was composed of fast and slow components (Goodford, 1964). The quantity of rapidly exchanging ^{42}K was also found to be in excess of that in the extracellular space (Goodford, 1966, 1967). This observation has also been reported for the uterus (Daniel, 1963).

Studies of the competitive characteristics of the fast ^{42}K exchange led Goodford to conclude that the fast exchanging ions might be associated to fixed anions on the cell surface (Goodford, 1966, 1967). He noted that the total amount of fixed charge (3 meq/kg wet wt or about 15 meq/kg d.s.) could be accommodated on a condensed lipid film (Goodford, 1967). Recent studies on sodium exchange in taenia coli indicate that 12 meq/kg wet wt or about 60 meq/kg d.s. exchanged according to bulk diffusion kinetics and was in excess of that distributed in the extracellular water (Brading and Jones, 1969). Although a surface layer of fixed negative charges interacting with external ions may account for much of the electrical behavior of smooth muscle, such a component cannot account for all the fast exchanging electrolyte.

On the basis of the evidence available it appears that vascular smooth muscle contains both fast and slowly exchanging electrolyte. It is not yet possible, however, to establish the presence or absence of a membrane transport process, capable of limiting the exchange of ions in this tissue.

The problem arises as to how vascular smooth muscle maintains a relatively low water, sodium, and chloride content under conditions in which much of the sodium and chloride exchange so rapidly as to be indistinguishable from that of the extracellular space. Moreover, there is the question as to how fast and slowly exchanging fractions of cell electrolyte can be maintained within the same cell membrane. A solution may lie in an approach which emphasizes both surface and bulk properties of smooth muscle rather than membrane properties alone.

This approach has been taken in smooth muscle physiology. Meigs after investigating the osmotic behavior of frog stomach smooth muscle concluded that it behaved more like a gel than a fiber surrounded by a semipermeable membrane (Meigs and Ryan, 1912; Meigs, 1912). Measurements of electrolyte movements indicated to Meigs that although much of the smooth muscle sodium was permeative, considerable amounts of potassium and magnesium were present in a nondiffusible form. Bozler expanded this approach in a series of investigations upon frog stomach muscle (Bozler et al., 1958; Bozler and Lavine, 1958; Bozler, 1959, 1961, 1962, 1965).

In general it was concluded that smooth muscle behaved like a gel in which the volume was maintained by elastic forces within a cross-linked macromolecular framework (Bozler, 1962, 1965). According to this view, differences in permeability to molecules of various sizes was the result of narrow channels within the fiber matrix (Bozler, 1959, 1965). In one of the earlier investigations it was suggested that a large amount of the intracellular potassium may be immobilized (Bozler et al., 1958). This aspect of the model was not expanded, however, in later work.

A unified theory of cellular behavior has been formulated by Ling which was based on the physicochemical properties of protein, ions, and water in biological systems (Ling, 1962). It would be appropriate to examine the present experimental findings in the light of this approach.

The heterogeneous exchange of electrolyte in vascular smooth muscle could be the result of two equilibrium states being available: (1) dissolved in the interstitial cell water contained within the protein matrix; (2) associated to charged groups fixed to the protein matrix distributed throughout the smooth muscle cell.

The dissolved electrolyte can be considered to be that quantity which followed bulk diffusion kinetics. Because of the high extracellular concentration, sodium and chloride are the predominant ions in this phase. It is possible that these ions undergo restriction, especially rotational and configurational (Ling and Ochsenfeld, 1966), but because of the extracellular diffusion delays a wide range of radial diffusion coefficients for the muscle cells would be compatible with the present results. This problem will have to be overcome before a reasonable estimate of the restriction to radial diffusion can be made. The partition coefficient for the distribution of dissolved electrolyte between intracellular and extracellular water can, however, be estimated from the experimental data. An estimate of the interstitial sodium, $[Na]_i$, concentration in PSS, 37°C yielded a minimum value of 34 meq/kg "cell" H_2O. In this calculation the total sodium (Table I) was corrected for slowly exchanging sodium (Table IV), mucopolysaccharide sequestered sodium, and that dissolved in the sucrose space. The concentration of chloride in the interstitial water, $[Cl]_i$ (corrected for slowly exchanging chloride and that dissolved in the sucrose space), was estimated to be 30 meq/kg "cell" H_2O. The partition ratio for sodium and chloride was 0.24 $[Na]_i/[Na]$out or $[Cl]_i/[Cl]$out. This value was slightly greater than that estimated for frog sartorius (Ling, 1966; Ling and Ochsenfeld, 1966).

The associated electrolyte can be considered to be that quantity which followed the reversible reaction kinetics. Under physiological conditions potassium was the principal cation associated with the fixed charge. However, in potassium-free medium at 37°C, potassium was replaced by a similar amount of associated sodium. These findings emphasized the reciprocal nature of the sodium-potassium exchange on the sites, with potassium being selectively taken up. Under inhibited conditions at 37°C little selectivity was observed, and the association of ions to the sites was weak, as indicated by the lack of slowly exchanging sodium or potassium. The selectivity at 37°C depended upon the availability of products from an active energy metabolism, e.g., ATP. Compounds such as ATP are considered in the model to control the selectivity of the fixed charges by adsorbing onto controlling or cardinal sites (Ling, 1962). The net loss of ATP by hydrolysis would therefore lead to the loss of selective accumulation. Under cold conditions (PSS, 2°C) the tissues exhibited little selectivity; however, rather strong association of ions to the fixed charges could be inferred from the presence of slowly exchanging sodium and potassium.

The observation that the rate of potassium exchange at 37°C may follow a distributed function is consistent with the current approach. In both skeletal and cardiac muscles it has been noted that the distribution of cell diameters and therefore available membrane area for each cell is insufficient to account for the standard deviation required to fit the potassium efflux curves (Creese et al., 1956; Persoff, 1960). Although similar anatomical studies have not been completed on vascular smooth muscle, it is anticipated that the flux variation does not result from a distribution of cell sizes or of cell types, but from intracellular factors, e.g., a distribution of adsorption energies for the sites on different proteins.

The maintenance of a relatively low cell volume under metabolically active conditions was thought to result from the maintenance of cross-links in the protein matrix (Ling, 1962). The swelling of the smooth muscle induced by cold incubation was considered to result from the breaking of cross-links, especially of the salt linkage form (carboxyl-amino association). Under these conditions the total slowly exchanging cation was greater than in PSS, 37°C. However, the slowly exchanging chloride increased by an equivalent amount, thereby balancing the charge. The increase in associated cation and anion at 2°C was taken as evidence that salt linkages between cationic and anionic side chains of protein had been broken. As in the case of ion-protein association, the availability of bioregulants such as ATP appeared to be necessary for a high degree of cross-linkage formation. This was indicated by the increased water content under metabolically inhibited conditions at 37°C.

The finding that vascular smooth muscle lost water, sodium, and chloride when transferred from potassium-free solution at 2°C to PSS−K, 37°C indicated that there was not an obligatory coupling between volume control processes and sodium-potassium exchange processes in the arterial wall. The relegation of volume control

processes to the associational interaction of fixed cationic and anionic groups in the protein, and the sodium-potassium exchange to the associational interaction between fixed anionic groups and interstitial cation, does not create an obligatory coupling between these two processes. The observed uncoupling is especially difficult for any membrane model which incorporates a sodium-potassium coupled transport mechanism.

The context in which the observations presented in this paper were placed represented a divergence from commonly applied membrane types of models. The characteristics of the ion exchange and volume control mechanisms operating in the arterial wall indicated that an extensive modification of any membrane model was needed, or that a new approach should be taken in order to interpret the present experiments and to design future ones. The latter course was chosen. As reported in the following paper, further investigations of the ion exchange properties have shown that the arterial wall exhibits saturation behavior with respect to the accumulation of ions, "cooperative" interaction between sodium and potassium accumulation, and competitive inhibition of potassium accumulation by rubidium (Jones and Karreman, 1969). These are all properties of a fixed-charge system and give further credence to the applicability of the association-induction hypothesis to the ion exchange properties of the arterial wall.

APPENDIX

The numerical analysis and computer programs for the solution of the partial and ordinary differential equations of the model of Crank (1956) used in the text,

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - \frac{\partial S}{\partial t} \tag{1}$$

$$\frac{\partial S}{\partial t} = \lambda C - \mu S, \tag{2}$$

or, more general, for the reaction order, $m > 1$,

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - \frac{\partial S}{\partial t} \tag{a}$$

$$\frac{\partial S}{\partial t} = \lambda C^m - \mu S \tag{b}$$

with

$$C = S = 0 \quad \text{for} \quad t < 0 \quad \text{and} \quad -a \leq x \leq a \tag{3}$$

and

$$C(\pm a) = pC_0 \quad \text{for} \quad t > 0, \tag{4}$$

in which C_0 is the external concentration of the isotope and p the partition ratio, has been made after the following transformations:

$$C = cpC_0 \tag{5}$$

$$S = spC_0 \tag{6}$$

$$x = ay \tag{7}$$

$$\tau = \frac{D}{a^2} t. \tag{8}$$

This yields the equations

$$\frac{\partial c}{\partial t} = \frac{\partial c}{\partial y^2} - \frac{\partial s}{\partial t} \tag{9}$$

$$\frac{\partial s}{\partial t} = \frac{a^2 \mu}{D} \left[\frac{\lambda}{\mu} (pC_0)^{m-1} c^m - s \right] \tag{10}$$

with

$$c = s = 0 \quad \text{for} \quad \tau < 0 \quad \text{and} \quad -1 \leq y \leq 1 \tag{11}$$

and

$$c(\pm 1) = 1 \quad \text{for} \quad \tau \geq 0. \tag{12}$$

The numerical analysis of equations (9) through (12) has been performed by the method of Crank and Nicolson (J. Crank and P. Nicolson. 1947. *Proc. Cambridge Phil. Soc.* **43**:50). In the case $m \neq 1$ a predictor-corrector method has been applied in addition.

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